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# The TRPA1 channel and oral hypoglycemic agents

## Is there complicity in β cell exhaustion?

Carlos Manlio Diaz-Garcia

Departamento de Neurodesarrollo y Fisiología; División de Neurociencias; Instituto de Fisiología Celular; Universidad Nacional Autónoma de México; Coyoacán, México DF, México

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Correspondence to: Carlos Manlio Diaz-Garcia; Email: cmanlio@email.ifc.unam.mx

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**Diabetes mellitus type 2 (DM2) results from the combination of insulin unresponsiveness in target tissues and the failure of pancreatic β cells to secrete enough insulin.[1] It is a highly prevalent chronic disease that is aggravated with time, leading to major complications, such as cardiovascular disease and peripheral and ocular neuropathies.[2] Interestingly, therapies to improve glucose homeostasis in diabetic patients usually involve the use of glibenclamide, an oral hypoglycemic drug that blocks ATP-sensitive $K^+$ channels ($K_{ATP}$),[3,4] forcing β cells to release more insulin to overcome peripheral insulin resistance. However, sulfonylureas are ineffective for long-term treatments and ultimately result in the administration of insulin to control glucose levels.[5] The mechanisms underlying β-cell failure to respond effectively with glibenclamide after long-term treatments still needs clarification. A recent study demonstrating that this drug activates TRPA1,[6] a member of the Transient Receptor Potential (TRP) family of ion channels and a functional protein in insulin secreting cells,[7,8] has highlighted a possible role for TRPA1 as a potential mediator of sulfonylurea-induced toxicity.**

Some members from TRP family of ion channels have been related to the cationic non-selective currents in pancreatic β cells.[9] TRPA1, a polymodal receptor that responds to noxius cold (< 17 °C), divalent cations ($Ca^{2+}$ and $Zn^{2+}$), electrophilic compounds,[10,11] and to polyunsaturated fatty acids,[12] allows for $Ca^{2+}$ permeation into the cells where it is expressed. High expression of the TRPA1 channel has been shown in rat pancreatic islets and short-term treatments (≤ 1 h) with the agonists 4-hydroxy-2-nonenal (4-HNE), allylisothiocyanate (AITC) and 15-deoxy-Δ12,14-prostaglandin $J_2$ result in a dose-dependent increase of cytosolic $Ca^{2+}$ with subsequent insulin release in RINm5F cells.[7] Similar results have been reported for native β cells using the above mentioned agonists, as well as with $H_2O_2$ and methylglioxal (MG), suggesting that TRPA1 could be the link between inflammatory signals and oxidative metabolism and insulin secretion.[8]

In a recent article published in the *European Journal of Pharmacology*, Babes and colleagues[6] reported that glibenclamide acts as an agonist of the TRPA1 channel. The authors demonstrated that this sulfonylurea, but not tolbutamide, induces intracellular $Ca^{2+}$ transients in a dose-dependent fashion and increases cationic non-selective currents in HEK293 cells expressing human TRPA1 (hTRPA1). Moreover, these effects could be reversed by specific TRPA1 antagonists, and the authors also identified 3 critical cysteine residues at positions 621, 641, and 665 in the N-terminus for glibenclamide-mediated activation. Finally, they observed that glibenclamide increased intracellular $Ca^{2+}$ in a subpopulation of dorsal root ganglion (DRG) neurons (9% of the sample when applied at a concentration of 200 μM), although native channels from mice show a decreased affinity as compared with the heterologously expressed hTRPA1.

These results, as discussed in their paper, support a role for TRPA1 in some

of the glibenclamide-related side effects in diabetic patients, such as the development of abdominal pain and of a hyperactive bladder.[6,11] In light of previous studies reporting TRPA1 expression in β cells, Babes and coworkers also suggested the potential participation of TRPA1 in the effects of glibenclamide as a secretagogue. This is not unlikely, since TRPA1 can promote insulin secretion in RINm5F cells when stimulated with the TRPA1 agonist AITC in the presence of the $K_{ATP}$ activator diazoxide, resulting in an increase of 35.5% compared with insulin secretion in the presence of diazoxide alone.[8] However, the therapeutic impact of this phenomenon does not seem to be completely straightforward since it has been reported that treatment with glibenclamide can be rendered completely ineffective in patients carrying a mutation in the KCNJ11 (Kir 6.2) gene.[13] This suggests that the possible "beneficial" activation of TRPA1 would not be able to overcome the glibenclamide resistance of $K_{ATP}$ channels to rescue a damaged glucose homeostasis. On the other hand, the results of Babes et al. shed light on the potential deleterious effects of long-term stimulation of TRPA1 channels by glibenclamide in patients with DM2.

Activation of TRPA1 by MG induces $Ca^{2+}$ transients and augments non-selective cationic currents in hTRPA1-transefected HEK293 cells and mouse sensory neurons.[14] In the same study, it was demonstrated that incubation with MG (10 mM for 20 min) reduces conduction velocity and amplitude of compound action potentials from wild-type but not from $TRPA1^{-/-}$ mice, which could be relevant per se in the development of diabetic neuropathy. Beta-cell damage by glibenclamide is also a well-documented event and there is an obvious concern about a potential interaction of glibenclamide with TRPA1 in this toxicity. Glibenclamide is known to induce oxidative stress and cell death in a dose-dependent manner in the insulinoma MIN6, exhibiting potencies higher than other anti-diabetic drugs (i.e., glimepiride, gliclazide and nateglinide).[15] Continuous exposure of the same cell line to oral hypoglycemiants (72 h), finally leads to decreased insulin secretion in response to an acute stimulus with sulfonylureas with reduced insulin content, downregulation of $K_{ATP}$ channels and increased apoptosis.[16] Similar results have been reported in human islets, regarding apoptotic death and impairment of insulin secretion after sustained application of glibenclamide for up to 4 d.[17]

It is worth noting that incubation with glibenclamide (100 μM for 8 h) also decreases the viability of RINm5F cells by almost 40% and the apoptotic events leading to cell death can be attenuated by reducing the extracellular $Ca^{2+}$ or applying nitrendipine.[18] Interestingly, long-term incubation (24 h) with the TRPA1 agonist 4-HNE also reduces the responsiveness of insulin-secreting cells and the expression of PDX-1 (a transcription factor that promotes insulin gene expression)[7] which indicates a TRPA1-mediated disruption of the β-cell physiology. Furthermore, impaired insulin secretion resulting from the $Ca^{2+}$ overload after continuous activation of TRPA1 could be potentiated by the inactivation of voltage-gated $Na^{+}$ channels due to an increased and sustained background depolarization, which is likely to occur in neurons.[14]

The actions of glibenclamide in β cells could be further enhanced by the complex etiology of DM2. This metabolic disease is characterized by an increase in inflammatory signals, circulating lipids and reactive oxygen species production, as well as other environmental pollutants[2,19] that may sensitize or act synergistically to activate TRPA1. There are some arguments that glibenclamide may not be responsible for β-cell failure, since incubating INS-1 cells with various concentrations of glibenclamide during 24 h, in a glucolipotoxic environment, did not cause a significant increase in apoptosis.[20] However, this observation contrasts with an overwhelming amount of evidence pointing to glibenclamide as a key player of β-cell exhaustion. More studies are needed to disregard possible differences between insulinomas and primary β cells, as well as differences among cell lines, but mainly to understand the complexity of the effectors at play in DM2.

In conclusion, the study of Babes and colleagues has identified glibenclamide as a novel activator of TRPA1, providing insights into some adverse effects observed in diabetic patients treated with this drug. This article supports the presence of another pathway responsible for the insulinotropic actions of glibenclamide, since both inhibition of $K_{ATP}$ channels and activation of TRPA1 could result in increased insulin secretion. Moreover, the results reported in this study could explain the initial exacerbation of hyperinsulinism in glibenclamide-treated DM2 patients, but also the progressive deterioration of β-cell function.

### Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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